Isolation of Novel Glucosides Related to Gingerdiol from Ginger and Their Antioxidative Activities

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Two novel glucosides of 6-gingerdiol were isolated from fresh ginger (*Zingiber officinale* Roscoe). Their structures were determined as 1-(4-*O*-*β*-D-glucopyranosyl-3-methoxyphenyl)-3,5-dihydroxydecane (**1**) and 5-*O*-*β*-D-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decane (**2**) by HRFAB-MS and NMR analyses, and the absolute configurations of both aglycons were identified as (*3S*,*5S*) by a comparison with synthetic compounds. After incubating these glucosides with acetone powder prepared from fresh ginger, they were confirmed to have been hydrolyzed to 6-gingerdiol by HPLC. This result suggests that these glucosides are the precursors or intermediates of 6-gingerdiol. To recognize their function, their antioxidative activities were investigated and compared to that of their aglycon, 6-gingerdiol, by a linoleic acid model system and by their DPPH radical-scavenging ability. Although **1** did not indicate any activity, **2** had as strong activity as the aglycon in both measurements.

Keywords: *Ginger (Zingiber officinale Roscoe); 6-gingerdiol; 6-gingerdiol glucoside; antioxidant; 1-(4-O-β-D-glucopyranosyl-3-methoxyphenyl)-3,5-dihydroxydecane; 5-O-β-D-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decane*

INTRODUCTION

Ginger, having a pleasant aroma and pungency, is one of the most popular spices and is used for cooking or medicine in its fresh or dried form. The physiologically active functions of spices have aroused strong interest during the last two decades, and many researchers have reported on the active compounds and their activities in vitro and in vivo (Okuda, 1997; Plumb et al., 1996; Ohtera, 1997; Deans and Ritchie, 1987). Ginger and the components in it are also known to possess such physiological features as antimicrobial, antioxidative, antitumor, and antiplatelet aggregation activities (Yin et al., 1998; Lee and Ahn, 1985; Kawamura and Kato, 1988; Conner, 1991; Kawakishi et al., 1994). In the case of foods, antioxidative activity is valuable because it prevents the off-flavor generated by the oxygenated degradation of lipids. In addition, antioxidative activity has recently become of more concern since it has been suggested to be connected with the inhibition of DNA damage, carcinogenesis, arteriosclerosis, and the aging process (Dutchie et al., 1997; Noroozi et al., 1998; Newmark, 1996; Aust, 1997; Yu et al., 1998). Phenolic compounds including flavonoids are known to be major antioxidative compounds in various herbs and spices (Carrubba et al., 1998; Nakatani, 1996). In regard to ginger, 6-gingerol and 6-shogaol, the characteristic pungent compounds of ginger, have been described as main antioxidants by Lee and Ahn (1985), and Kikuzaki et al. have also reported more gingerol-related compounds and diarylheptanoids from dried ginger (1991, 1992, 1993). On the other hand, the phenolic compounds in plants are generally known to be generated from such phenolic amino acids as phenylalanine and tyrosine; moreover, the glycosylation of such phenolic compounds has been developed as precursors, intermediates, or metabolites for biosynthesis. We have recently isolated some glucosides of terpene alcohols as aroma precursors from fresh ginger and confirmed that they were hydrolyzed by endogenous glycosidase in a crude enzymatic system (Sekiwa et al., 1999). Therefore, such antioxidative phenolic compounds as those related to gingerol are also presumed to be formed from their glycosides as precursors. However, there have been no reports on detecting such phenolic glycosides in ginger.

In this study, we isolated and identified the glucosides of 6-gingerdiol, which is related to 6-gingerol, and confirmed its formation from glucosides by a crude enzymatic system with acetone powder from fresh ginger. Furthermore, their functions as antioxidants were examined in a model system of linoleic acid and by measuring their DPPH radical-scavenging activities.

MATERIALS AND METHODS

Plant Material. Rhizomes of fresh ginger (*Sinshoga, Z. officinale* Roscoe) harvested in Mie prefecture in Japan were obtained from a local market.

Extraction and Isolation of the Glucosides. Fresh rhizomes (3 kg) of ginger were homogenized in methanol (9 L) and extracted at 4 °C for 1 day. After filtration, the residue was extracted twice more by the same procedure. Methanol was evaporated from the combined extract, and the concentrated aqueous solution was subjected to column chromatography with Amberlite XAD-2 resin. The column was washed with distilled water, and the free volatiles were eluted with pentane/ether (4:6). The glycosidically bound fraction was obtained by eluting with methanol (4 L) and concentrated. Since some free phenolic compounds were included in this fraction, they were removed with chloroform by SiO_2 column

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chromatography, before the glycosides were eluted with methanol, and then concentrated. This concentrate (1.8 g) was subjected to flash column chromatography on ODS resin and fractionated by stepwise elution with a 10, 20, 30, 40, 50, 70, 80, and 100% methanol aqueous solution. After concentrating to dryness under reduced pressure, the 50% methanol eluate (274 mg) was further fractionated and purified by preparative high-performance liquid chromatography (HPLC), using a PEGASIL ODS column (Senshu Scientific Co., Tokyo, Japan; column size ϕ 20 × 250 mm) and a UV detector (210 nm). Compounds **1** (7.8 mg) and **2** (12.0 mg) were isolated by gradient elution with a CH_3CN/water system.

Spectral Analyses. ^1H and ^{13}C NMR spectra were obtained with JEOL-JNM-GX-270 (270 MHz) and FTNMR spectrometers, and the HMBC experiment was done with a JEOL-JMN-AL-300 (300 MHz) spectrometer. Each isolated glucoside was dissolved in CD_3OD containing TMS as an internal standard. FAB-MS data were measured with a JEOL MStation JMS-700 instrument with glycerin as the matrix. Optical rotation data were measured by a JASCO Dip-1000 instrument.

*1-(4-O-β-D-Glucopyranosyl-3-methoxyphenyl)-3,5-dihydroxydecane (**1**):* amorphous powder; HRFAB-MS *m/z* 457.2428 [M − H]$^-$ (error mmu = −0.9 for $C_{23}H_{38}O_9$); specific rotation $[\alpha]_D^{24.2}$ −29.3 (*c* 0.69, MeOH); ^1H NMR (in CD_3OD, 270 MHz, *J* in Hz) δ 0.91 (3H, t, *J* = 6.3, H-10), 1.32 (6H, m, H-7, 8, 9), 1.43 (2H, m, H-6), 1.49 (1H, dd, *J* = 2.0, 4.2, H-4a), 1.52 (1H, dd, *J* = 3.0, 5.3, H-4b), 1.71 (2H, td, *J* = 6.8, H-2), 2.60 (1H, ddd, *J* = 7.0, 7.5, 13.8, H-1a), 2.72 (1H, ddd, *J* = 7.0, 7.6, 13.5, H-1b), 3.36−3.49 (4H, m, H-2″, 3″, 4″, 5″), 3.67 (1H, dd, *J* = 4.9, 12.2, H-6″a), 3.82 (1H, m, H-6″b), 3.84 (3H, s, O−CH_3), 3.84 (1H, m, H-5), 3.88 (1H, m, H-3), 4.82 (1H, d, *J* = 7.3, H-1″), 6.74 (1H, dd, *J* = 1.6, 8.3, H-6′), 6.86 (1H, d, *J* = 2.0, H-2′), 7.06 (1H, d, *J* = 8.3, H-5′).

*5-O-β-D-Glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decane (**2**):* colorless oil; HRFAB-MS *m/z* 457.2444 [M − H]$^-$ (error mmu = +0.7 for $C_{23}H_{38}O_9$); specific rotation $[\alpha]_D^{23.9}$ −13.3 (*c* 0.72, MeOH); ^1H NMR (in CD_3OD, 270 MHz, *J* in Hz) δ 0.90 (3H, t, *J* = 7.0, H-10), 1.33 (6H, m, H-7, 8, 9), 1.50 (2H, m, H-6), 1.59−1.64 (2H, m, H-4), 1.65−1.72 (2H, m, H-2), 2.56 (1H, m, H-1a), 2.68 (1H, m, H-1b), 3.17 (1H, t, *J* = 8.3, H-2″), 3.25−3.38 (3H, m, H-3″, 4″, 5″), 3.68 (1H, dd, *J* = 5.2, 11.9, H-6″a), 3.84 (1H, m, H-6″b), 3.83 (3H, s, O−CH_3), 3.92 (1H, m, H-3), 3.92 (1H, m, H-5), 4.39 (1H, d, *J* = 7.6, H-1″), 6.62 (1H, dd, *J* = 2.1, 6.1, H-6′), 6.68 (1H, d, *J* = 7.9, H-5′), 6.77 (1H, d, *J* = 1.9, H-2′).

Synthesis of Compound 1. To identify the structure of compound **1**, authentic **1** was synthesized from α-D-acetobromoglucose and authentic 6-gingerol by the method for selectively binding to the phenol group (Mulkens and Kapetanidis, 1988). 6-Gingerol had already been synthesized by the method of Wada et al. (1996). 6-Gingerol (2.0 mmol) and 5.0 mmol of α-D-acetobromoglucose were dissolved in 20 mL of dehydrated acetone, and the mixture stirred for 5 h. This reaction was maintained at a pH value higher than 8.0 by dropwise addition of a 0.725 N KOH aqueous solution while stirring. The reaction products were extracted with benzene and concentrated. After purification by SiO_2 column chromatography with ethyl acetate/hexane, the acetylated glucoside, 5-hydroxy-1-(4-*O*-tetra-*O*-acetyl-β-D-glucopyranosyl-3-methoxyphenyl)decan-3-one, was obtained in a yield of 1.1 mmol. This acetylated glucoside (0.93 mmol) was reduced with $LiAlH_4$ in dehydrated THF, and 0.68 mmol of **1** was obtained (32% yield).

Determination of the Absolute Configuration of the Aglycon in Each Glucoside. The absolute configuration of the aglycon in each glucoside was measured after hydrolyzing with glycosidase (Rohapect D5L; Rohm, Darmstadt, Germany). Each enzymatically reacted solution was passed through a column of XAD-2 resin to extract the free liberated 6-gingerdiol with methanol and then subjected to preparative HPLC. The absolute configuration of each sample was determined by comparing its NMR data to those of synthetic (3*S*,5*S*)- and (3*R*,5*S*)-6-gingerdiol prepared according to the method of Kikuzaki et al. (1992).

Hydrolysis of the Isolated Glucosides by Acetone Powder from Ginger. Acetone powder was prepared by the same method as that described in the previous report (Sekiwa et al., 1999); 2 mg of each isolated glucoside was dissolved in 20 mL of a 50 mM citrate buffer (pH 6.0). After 1.0 g of acetone powder had been added, each solution was incubated for 19 h at 37 °C. The reacted solutions were centrifuged (3400 rpm × 20 min, 4 °C), and the resulting supernatants were applied to an XAD-2 column, extracted with methanol, and subjected to HPLC analysis.

HPLC Analysis of the Hydrolyzed Samples. HPLC analysis of each hydrolyzed sample was performed by a Waters 600E instrument connected to a Waters 996 photodiode array detector with PEGASIL ODS (Senshu Scientific Co., Tokyo, Japan; column size ϕ 4.6 × 250 mm). The solvent system was set in a gradient from 30% CH_3CN/water to 80% for 100 min, and the flow rate was 1.0 mL/min. The HPLC chromatogram was monitored at a UV wavelength from 190 to 340 nm.

Antioxidant Assay on the Model System with Linoleic Acid Autoxidation. The antioxidative activity with linoleic acid was measured by the method of Inatani et al. (1983). A 5.1 μmol sample of each compound was added to a 2.5% linoleic acid solution consisting of 99.5% ethanol (8.1 mL), 50 mM phosphate buffer (pH 7.0, 8.0 mL), and purified water (3.9 mL) in a screw-top vial (36 mm i.d. × 75 mm). A solution without the antioxidant sample was used as a control. Duplicate vials were prepared for each sample. Each vial was incubated at 45 °C for 20 days in the dark and measured every 5 days. The hydroperoxide and aldehyde generated from linoleic acid in each sample solution were determined by the thiocyanate and thiobarbituric acid (TBA) methods, respectively. After an aliquot (100 μL) of the reaction solution had been mixed with 75% ethanol (9.7 mL) and 30% ammonium thiocyanate (100 μL), 20 mM $FeCl_2$ (100 μL) was added and the mixture was vigorously shaken. The generated color was measured by the absorbance at 500 nm after 3 min. Sample solutions after the first day and last day of incubation were each subjected to the TBA method. Two milliliters of the reaction mixture was mixed with 20% trichloroacetic acid (2.0 mL) and 0.67% TBA/99.5% ethanol (1.0 mL) and the mixture heated in a boiling-water bath for 10 min. After cooling and centrifugation (3000 rpm, 15 min), the generated TBA reactive substances were measured by their absorbance at 532 nm.

Measurement of DPPH Radical-Scavenging Activity. The effect of each sample on the DPPH radical was determined by the method of Yamaguchi et al. (1998). Each antioxidant was dissolved in ethanol at various concentrations. An aliquot of the sample solution (300 μL) was mixed with a 100 mM Tris-HCl buffer (pH 7.4, 1200 μL). After this solution had been added to 500 μM DPPH/95% ethanol (1500 μL) and shaken vigorously, it was left to stand for 20 min in the dark and then measured for its color at 517 nm. A control was prepared by adding ethanol instead of the sample solution. The DPPH radical-scavenging activity of each sample was evaluated by comparing it to the control value.

RESULTS AND DISCUSSION

Structures of Glucosides 1 and 2. Repeated separation by flash column chromatography and preparative HPLC of the crude glycosidically bound fraction enabled two phenolic glucosides to be isolated. The structures of isolated glucosides **1** and **2** were analyzed by NMR and mass spectroscopy. The ^{13}C NMR spectra are shown in Table 1. The molecular formula of compound **1** indicated $C_{23}H_{38}O_9$ from measurements by HRFAB-MS. The signal patterns of the aglycon of **1** by ^1H and ^{13}C NMR were close to those of 6-gingerol. However, the signal at δ 68.6 indicating C-3 on compound **1** was observed at a higher field than that of 6-gingerol by ^{13}C NMR, this being correlated to the signal at δ 3.88 from ^1H NMR by a $^{13}C-^1H$-COSY analysis. Therefore, the aglycon of **1** seemed to be 6-gingerdiol, which has the reduced form of 6-gingerol at C-3. On the other hand, the ^1H NMR signals at δ 3.36−3.67 and the character-

Table 1. ^{13}C NMR Spectral Data for Compounds 1 and 2[a]

	compd **1**	compd **2**		compd **1**	compd **2**
1	32.7	32.7	3′	150.5	148.8
2	41.2	41.3	4′	145.9	145.4
3	68.6	68.2	5′	118.1	116.1
4	45.7	42.9	6′	121.8	121.7
5	69.2	78.8	1″	103.0	104.4
6	39.2	36.9	2″	74.9	75.5
7	26.5	26.0	3″	78.1	78.2
8	33.1	33.1	4″	71.3	71.6
9	23.8	23.7	5″	77.8	77.8
10	14.5	14.4	6″	62.5	62.8
1′	138.7	135.4	OMe	56.3	56.3
2′	113.9	113.1			

[a] Measured in CD_3OD.

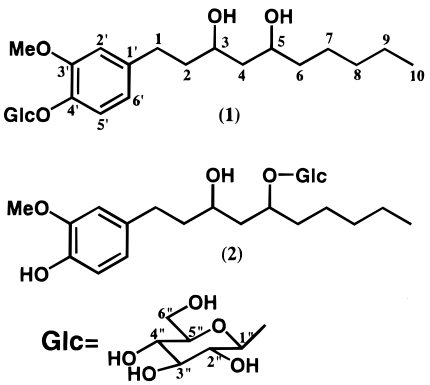

Figure 1. Structure of 6-gingerdiol glucosides, compounds **1** and **2**.

istic doublet signal (J = 7.3 Hz) at δ 4.82, indicative of an anomeric proton, revealed the sugar moiety as β-D-glucopyranoside, this being confirmed by the ^{13}C NMR data. By comparing the ^{13}C NMR data with those of authentic 6-gingerdiol, the small shifts of C-3′ and C-5′ on the phenyl group in compound **1** indicated glucose connected to −OH on the phenyl group (Table 1). Furthermore, the data for this compound agreed with those of the synthesized β-glucoside in which glucose was bound to the phenyl group of 6-gingerdiol. Therefore, compound **1** was concluded to be 1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)-3,5-dihydroxydecane (Figure 1).

The molecular formula of compound **2** was also $C_{23}H_{38}O_9$ by HRFAB-MS measurements. The patterns of the NMR spectra of compound **2** were similar to those of compound **1**, except for the doublet of an anomeric proton in glucose that was found at δ 4.39 by ^{1}H NMR and the signals of C-4, C-5, and C-6 shifted to δ 42.9, 78.8, and 36.9 by ^{13}C NMR (Table 1). Therefore, the structure of compound **2** also seemed to be the β-D-glucoside of 6-gingerdiol. Furthermore, the cross-peak between the signal of the anomeric proton (1″) and C-5 by HMBC analysis of compound **2** confirmed glucose connected to alcoholic −OH at the C-5 position. Therefore, compound **2** was determined to be 5-*O*-β-D-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-decane. 6-Gingerdiol is known as a characteristic compound in ginger together with 6-gingerol (Yamagishi et al., 1972; Masada et al., 1974). However, its glucosides were found for the first time in this study.

To determine the absolute configuration of the aglycon, each glucoside was hydrolyzed by glycosidase, and liberated 6-gingerdiol was analyzed by NMR measurements. Both liberated free 6-gingerdiols from compounds **1** and **2** indicated absolutely the same signal

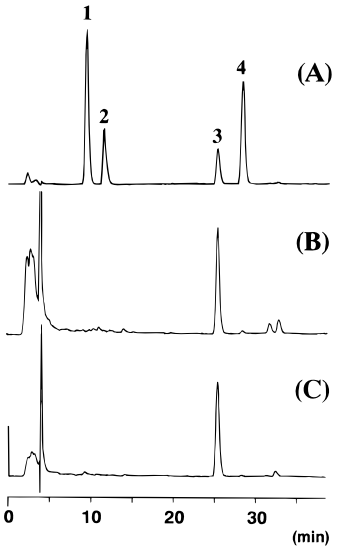

Figure 2. HPLC chromatograms of standard compounds (A) and compounds **1** fraction (B) and **2** fraction (C) after incubating with acetone powder. (1), (2), (3), and (4) refer to compound **1**, compound **2**, (3*S*,5*S*)-6-gingerdiol, and (3*R*,5*S*)-6-gingerdiol, respectively.

patterns. Furthermore, they agreed with those of synthesized (3*S*,5*S*)-6-gingerdiol. Therefore, the absolute configuration of the aglycons in compounds **1** and **2** was determined as (3*S*,5*S*).

Hydrolysis of the Glucosides by Acetone Powder Prepared from Fresh Ginger. After compounds **1** and **2** were incubated with the acetone powder of ginger, the liberated compounds were extracted and successively subjected to reversed-phase HPLC analysis. The HPLC chromatograms are shown in Figure 2. The retention times for compounds **1**, **2**, (3*S*,5*S*)-6-gingerdiol, and (3*R*,5*S*)-6-gingerdiol were 9.27, 11.40, 25.09, and 28.07 min, respectively. By adding the acetone powder to compounds **1** and **2**, the peak with the retention time and UV spectrum corresponding to (3*S*,5*S*)-6-gingerdiol was detected, and no peak indicating glucoside was apparent.

We have already reported that the glucosides of terpene alcohol were hydrolyzed with acetone powder from ginger (Sekiwa et al., 1999). Therefore, compounds **1** and **2** were also deduced to have been hydrolyzed by endogenous glycosidase in ginger, and these glucosides are suggested to be intermediates of 6-gingerdiol. Denniff and Whiting (1976) and Macleod and Whiting (1979) have reported the biosynthesis of 6-gingerol from phenylalanine and dihydroferulic acid. 6-Gingerdiol is also presumed to be generated along the same pathway, because of the similarity of their structures. To have detected the glucosides of 6-gingerdiol and endogenous glycosidase in this study might be of interest to clarify these formation mechanisms in ginger.

Antioxidative Activity of 6-Gingerdiol Glucosides. 6-Gingerdiol has already been found as an antioxidant in ginger (Kikuzaki et al., 1993). To determine the use and function of the isolated glucosides, their antioxidative activities were examined and compared to those of 6-gingerdiol and 6-gingerol. The result with the linoleic acid model system is shown in Figure 3. The results by both the thiocyanate and TBA methods indicated the same trend. Compound **1** showed almost the same value as the control and had no antioxidative

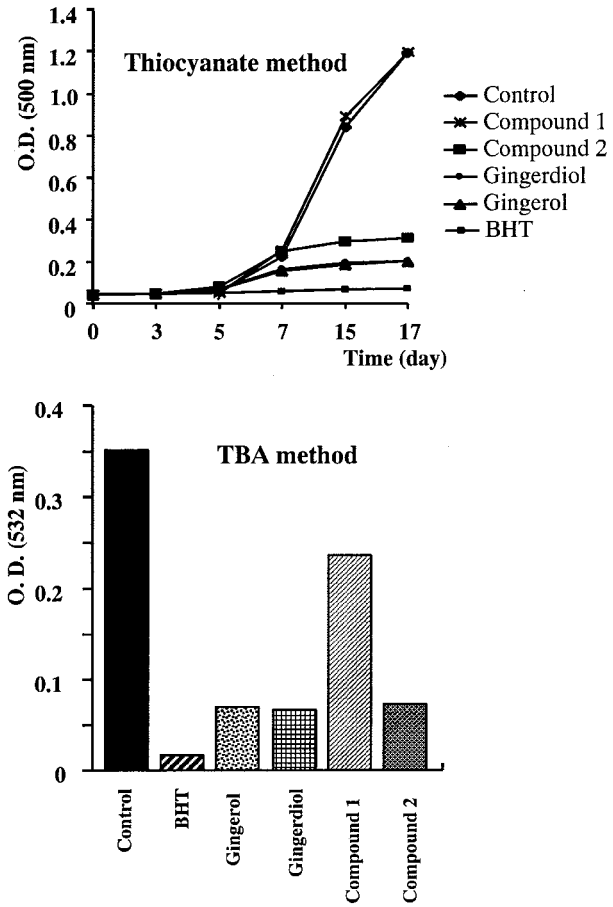

Figure 3. Antioxidative activity of compounds **1** and **2**, gingerdiol, gingerol, and BHT for linoleic acid. Control means no added antioxidant.

activity. Compound **2** had as strong activity as 6-gingerdiol and 6-gingerol, although being less active than BHT.

Figure 4 shows the DPPH radical-scavenging activity of the glucosides and other antioxidants. Compound **2**, 6-gingerdiol, and 6-gingerol more strongly scavenged the DPPH radical with increasing concentration and almost completely scavenged it at 100 μM. Moreover, their activities were almost equal to those of such typical natural antioxidants as α-tocopherol and ascorbic acid. On the other hand, compound **1** had no DPPH radical-scavenging activity. These results agree with those for the linoleic acid model system. Compounds **1** and **2** were different in the position of bound glucose, as already stated. The results of almost the same strong activity of compound **2** versus 6-gingerdiol and 6-gingerol mean that the alcoholic OH on decane, side chain of phenol, does not influence the antioxidative activity. In general, phenolic OH is known as a scavenger of free radicals and it consequently exhibits antioxidative activity. Especially, in regard to substitution on the phenyl ring, several studies have reported that the existence of an electron-donating group such as methoxy substitution in the position *ortho* to OH enhances antioxidant effectiveness (Rice-Evans et al., 1996; Toda et al., 1985; Cuvelier et al., 1992). Therefore, compound **2**, in which phenolic OH is free, is suggested to have strong antioxidative activity. On the other hand, phenolic OH was blocked by glucose in compound **1**. The results of no activity of this suggested the importance of free phenolic OH on the phenyl ring in gingerdiol for antioxidative activity. Kikuzaki (1996) revealed that dimethoxy substitutions of the phenyl ring in gingerol and shogaol weakened the antioxidative activity, and this is likely to support our results.

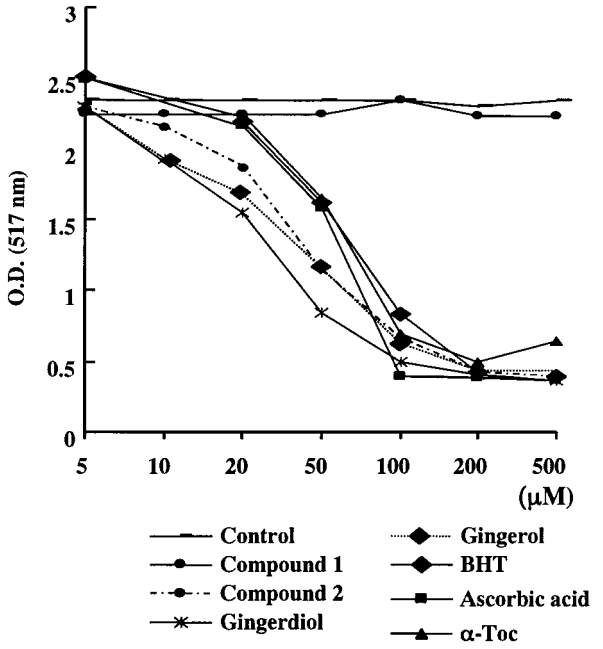

Figure 4. DPPH radical-scavenging activity of antioxidants. Control means no added antioxidant.

However, compound **1** is transferred to 6-gingerdiol as an antioxidant in such a specific condition as hydrolyzation of a glucoside. Glucosidic flavonoids have recently been reported to be enzymatically hydrolyzed during metabolism and adsorption, and the aglycon acted as an antioxidant in vivo (Miyake et al., 1997; Ioku et al., 1998). Therefore, compound **1** is suggested to also be useful as an antioxidant with compound **2** after hydrolyzation. Considering their low yields (6.5×10^{-3}% for **1**, 10.0×10^{-3}% for **2** in dry weight of ginger), their antioxidative activities as ginger might not be expected, but they are presumed to be useful as food ingredients and additives. Furthermore, 6-gingerdiol has other physiological activities, in addition to its antioxidative activity (Kimura et al., 1988; Yamahara et al., 1992). Since these glucosides act as intermediates of 6-gingerdiol, as already indicated, they are presumed to increase the quantity of 6-gingerdiol and then improve the functional quality of ginger.

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